

Neurobehavioral effects of methyl bromide inhalation exposures

by W Kent Anger, PhD, James V Setzer, BS, John M Russo, PhD, W Stephen Brightwell, BS, Robert G Wait, PhD, Barry L Johnson, PhD¹

ANGER WK, SETZER JV, RUSSO JM, BRIGHTWELL WS, WAIT RG, JOHNSON BL. Neurobehavioral effects of methyl bromide inhalation exposures. *Scand j work environ health* 7 (1981): suppl 4, 40—47. Methyl bromide, a neurotoxic agent with a permissible US exposure limit of 20 ppm, is used primarily as a fumigant by an estimated 75,000 workers in the United States. This project was developed to evaluate the neurobehavioral effects of chronic and subchronic exposure to methyl bromide. One group of rats and rabbits was exposed to 65 ppm of methyl bromide for a total exposure of four 25-h weeks, or 100 h, and rats were exposed to 55 ppm of methyl bromide for a total exposure of 36 30-h weeks, or 1,080 h. Comparable control groups were given similar treatment, but no exposure. Behavioral tests of open field activity and limb coordination were conducted weekly during both phases of the experiment with rats, and eyeblink reflexes were measured weekly in rabbits. Nerve conduction velocity measurements were taken weekly from both rats and rabbits during the 65-ppm four-week exposures and monthly from rats during the 55-ppm 40-week exposures. Exposure to 65 ppm for four weeks significantly reduced eyeblink responses and nerve conduction velocity in rabbits but had no effect on rats. Exposure to 55 ppm of methyl bromide for 36 weeks had no effect on nerve conduction velocity, open-field activity, or coordination in rats.

Key terms: fumigants, nerve conduction, neurotoxicity, pesticides, rabbits, rats.

Methyl bromide is a colorless, nearly odorless gas at room temperature that is readily absorbed through the lungs and, if ingested, the gastrointestinal tract (15). It is used principally as a fumigant of fruits and grains, although it is also used in refrigeration and fire extinguishing systems (4, 9, 16), by an estimated 75,000 workers in the United States. Occupational use of methyl bromide has resulted in at least five deaths since 1957 (7) and in numerous episodes of overexposure which required medical attention (2, 3, 6, 7, 12, 14, 18). Pulmonary edema is the chief danger in

acute poisoning, but a variety of neurological symptoms are also present. These symptoms include muscle weakness and pain, loss of coordination and gait, and inability to focus one's eyes. Psychological symptoms such as depression have also been described in some cases.

Chronic exposures to low concentrations of methyl bromide have also been reported, and, in these instances, the neurobehavioral symptoms were the primary effects. Following chronic exposure, tremor and loss of cutaneous sensation are found in addition to the problems reported after acute exposures, such as weakness, incoordination, and difficulty in focusing (5, 7, 12, 13). Existing exposure concentrations have been measured in few studies, and characterization of past exposure histories of the persons or job sites is entirely lacking. In the most thorough evaluation of low-level methyl bromide exposures, Watrous (17) reported concentra-

¹ Division of Biomedical and Behavioral Science, National Institute for Occupational Safety and Health (NIOSH), Cincinnati, Ohio, United States.

Reprint requests to: Dr WK Anger, National Institute for Occupational Safety and Health, 4676 Columbia Parkway, Cincinnati, OH 45226, USA.

tions below 35 ppm (using a gross measuring device, the halide detector) in a plant where ampuls were being filled with methyl bromide. Several workers reported anorexia and headache during the two-week course of the filling operation, and a few also reported vertigo, faintness or numbness, although these latter symptoms may have been associated with episodic exposures above 35 ppm.

Despite the apparent toxicity of this compound, only one laboratory study has been published using animal models to evaluate the degree of toxic effects of different concentrations following long-term exposures. Irish et al (10) exposed rats, rabbits, guinea pigs, and monkeys to various concentrations of methyl bromide for 5 d/week over a six-month period. Guinea pigs were unaffected by exposures to 100 ppm of methyl bromide, but rats lost weight and were subjectively judged to have a poor appearance at this concentration. Rats and guinea pigs were unaffected by exposures to 65 ppm, but rabbits became paralyzed and several died. Three of six monkeys developed paralysis at 65 ppm, while two appeared unaffected and one died of gastrointestinal problems. At 33 ppm, rats, guinea pigs, and monkeys appeared normal, but 34 of 58 rabbits (nearly 60 %) became paralyzed. Rabbits survived 17-ppm exposures with no obvious effects.

The American Conference of Governmental Industrial Hygienists (ACGIH's) (1) recommended threshold limit value (TLV) of 15 ppm (with an intended change to 5 ppm) for methyl bromide is based on the studies already outlined; the permissible federal standard is 20 ppm. The purpose of the present study was to evaluate chronic and subchronic exposures to methyl bromide with the use of more sensitive tests of neurotoxicity than were available in 1940 (10) when only gross changes such as paralysis were noted. Rats and rabbits were exposed to 65 ppm of methyl bromide for four 25-h weeks and tested for activity and coordination (rats only), eyeblink reflex (rabbits only), and nerve conduction changes (rats and rabbits). Rats were also exposed to 55 ppm of methyl bromide for 36 30-h weeks and tested as already presented.

Methods and materials

Subjects

Sixty Sprague Dawley rats supplied by Harlan Industries (Indianapolis, IN) and Charles River Laboratories (Wilmington, MA) and eight New Zealand White rabbits purchased from King's Wheel Rabbitry (Mt Vernon, OH) served as the subjects. The original suppliers of rats were replaced due to the unavailability of healthy animals. At the outset of the experiment, the mean weight of the rats was 366 g (SD $\pm$ 38) and 544 g (SD $\pm$ 35) from Harlan and Charles River, respectively, and the mean weight of the rabbits was 2,418 g (SD $\pm$ 199). The rats had access to Purina 5001 Rodent Laboratory Chow, and the rabbits were given Purina 5321 Lab Rabbit Chow (Ralston Purina, St Louis, MO). Food and water were available ad libitum when exposure was not in progress.

Chambers and exposures

The exposure chambers in which the animals were housed 24 h/d during the experiment were standard 0.5-m³ stainless steel exposure chambers (Charles Spengler & Associates, Cincinnati, OH) with a conical mixing plenum and exhaust area. The rats were housed in pairs in stainless steel wire mesh cages arranged in two tiers in the chambers, with a maximum of 16 rats per chamber. Chamber cleaning created a random rotation of pairings and cage placement. Rabbits were housed on either side of a mesh partition, placing two rabbits in each chamber.

Exhaust air exited the chambers (approximately one air change/min) and passed through charcoal filters to remove contaminants before being released into the outside air. Mixtures of 10 % methyl bromide/90 % air prepared from 99.9 % pure methyl bromide stock (AIRCO Industrial Gases, Riverton, NJ) was supplied in compressed gas cylinders at 95 psi (655,025 Pa) (Wright Bros, Cincinnati, OH). Methyl bromide was directed from the cylinders through Matheson 1H-540 regulators (Matheson Gas, East Rutherford, NJ), to 0.25-in (0.63-cm) teflon tubing, and finally through Matheson 7640 series flow meters for calibrated introduction into the

exposure chamber plenum where it was mixed with air for dilution to exposure concentrations of 55 and 65 ppm. Air samples were automatically drawn from each chamber every 12 min and processed through a Miran II infrared analyzer (Foxboro-Wilks, Norwalk, CT). The mean measured concentration was 52.9 (SD $\pm$ 5.6) ppm for the 55-ppm exposures. Rat exposures targeted at 65 ppm had a mean concentration of 64.9 (SD $\pm$ 5.2) ppm, and the mean of the rabbit exposures was 68.4 (SD $\pm$ 12.4) ppm. Room air was maintained at approximately 72°F (22°C) with infrequent excursions as high as 80°F (27°C) or as low as 65°F (18°C).

Protocol and tests

Rabbits and rats obtained from Charles River were exposed to targets of 0 ppm (4 rats; 2 rabbits) or 65 ppm (16 rats; 6 rabbits) of methyl bromide for four 30-h weeks (4 d/week, 7.5 h/d), yielding an actual exposure time of 100 h over a period of one month (a subchronic regimen), and neurobehavioral testing occurred weekly. In a separate set of exposures, rats obtained from Harlan Industries were administered concentrations of 0 (8 rats) or 55 (32 rats) ppm of methyl bromide vapor for a total of 36 30-h weeks (5 d/week, 6 h/d) or 1,080 h over a period of 12 months (a chronic regimen), and neurobehavioral tests were conducted at 25- to 30-d intervals during the exposure. This test schedule ensured that a minimum of one no-exposure day preceded the test sessions. In addition, tests were conducted prior to the initiation of exposure with all animals to provide a baseline period for comparison.

The neurobehavioral tests, administered variously to the rats and rabbits, consisted of the evaluation of (i) conduction velocity in the sciatic and ulnar nerves, (ii) eyeblink reflex of the rabbits, (iii) open field activity of the rats, and (iv) grip/coordination of the rats.

Nerve conduction. Motor nerve conduction velocities were derived from the electrical stimulation, through steel needle electrodes, of the proximal and distal segments of the sciatic and ulnar nerves with a 0.2-ms, positive-going, square wave

pulse provided by a Grass Model S88 biomedical stimulator (Grass Instruments, Quincy, MA) and associated isolation circuitry. The responses of the muscles innervated by the sciatic and ulnar nerves were amplified and displayed on a Tektronix 5441 storage oscilloscope (Tektronix, Dayton, OH), from which response latencies were recorded. All measurements were made with the animals under a 20 mg/kg (0.091 mmol/kg) dose (intramuscular) of xylaxine anesthesia. Details of these procedures have been reported by Johnson et al (11). Animal weights were recorded prior to these tests for a determination of dosage and for weight information during exposures.

Eyeblink reflex. Unanesthetized rabbits were individually restrained in a Plexiglas® enclosure located in a brightly illuminated, sound-attenuating chamber. After a brief period of acclimatization, eyeblink reflexes were elicited, at 7-s intervals, by an air puff directed to the right cornea until a total of 50 responses were obtained. Electromyographic activity of the orbicularis oculi muscle of the eye (which encircles the eye) ipsilateral to the reflex-eliciting stimulus was measured with needle electrodes inserted under the skin adjacent to the right temporal canthus and superior palpebral portions of the muscle. A ground electrode was placed at the base of the right pinna. The electrical activity measured in this manner was amplified by a Grass Instruments model 78B polygraph and then gated through a Beckman 9852A integrator (Beckman Instruments, Schiller Park, IL) to a PDP-12 computer (Digital Equipment Corporation, Dayton, OH) which recorded the peak integrated response amplitude in a 100-ms period after onset of the air puff. Response amplitudes, scored in microvolts, were then averaged for each rabbit to provide the measure of reflex strength.

Activity. Rats were tested in a typical open field apparatus consisting of a circular Plexiglas field 0.98 m in diameter surrounded by an opaque wall 0.5 m high. Rats were individually placed on the Plexiglas floor, which was divided into 19 segments of nearly equal area (300 cm²), and allowed to roam for 2 min. The num-

ber of segments in which the rat placed all four paws was recorded as the measure of activity.

Grip/coordination. Grip/coordination was tested by the placement of rats individually on a series of four angle irons, each oriented to form an inverted V in cross-section. Placement was such that the ventral surface of the rat touched the peaked midpoint of the angle iron and thus forced the rat to grip the edge of each side to prevent falling. The four angle irons had different side lengths (6.2 cm, 5 cm, 3.8 cm, and 2.6 cm) to provide gradations in difficulty. Rats were placed on each angle iron sequentially, from the iron with the longest side to the one with the shortest, and given approximately 5 s to grip the side. The number of paws gripping each angle iron was recorded as the measure of coordination.

Results

In the subchronic exposure regimen, rabbits exposed to 65 ppm of methyl bromide for four weeks declined in weight from a mean of 2,304 g in the baseline period to a mean of 2,244 g in the final week of exposure ($t = 0.4$, $p = 0.3$, fig 1). Informal observations indicated that the animals stopped using their hind limbs and failed to clean themselves normally during the fourth week of exposure. The control animals showed none of these signs but generally gained weight during the experiment, as would be expected for young adult rabbits.

There was a substantial decrease in mean sciatic and ulnar conduction velocity in the rabbits by the end of the fourth week of 65-ppm methyl bromide exposure (table 1). The mean sciatic conduction velocity declined by 11.1 m/s, and ulnar velocity by 12.0 m/s during the exposures, but the mean values of the controls did not change. Due to the small number of controls (2 vs 6 exposed rabbits), a paired t-test was run between the base line and the conduction velocities of the final week for the exposed subjects; the changes in both the sciatic and ulnar nerve measurements were significant ($t = 7.17$, $p = 0.001$ for sciatic; $t = 4.36$, $p = 0.01$ for ulnar). Sciatic conduction velocity is presented in fig 2.

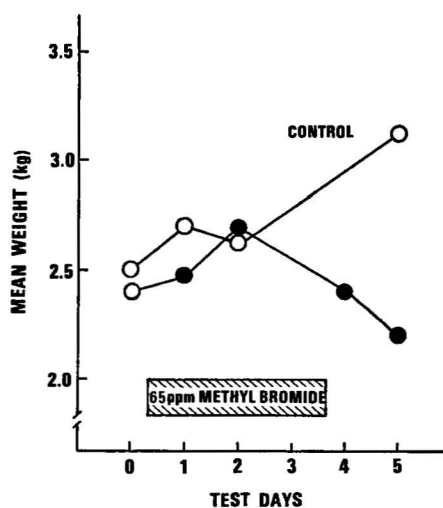


Fig 1. Mean weight of rabbits during the baseline period (week 0) and after exposure to 65 ppm of methyl bromide and of control rabbits over the same period of time.

Table 1. Conduction velocity (m/s) in the sciatic and ulnar nerves of rabbits and rats exposed to 0, 55, or 65 ppm of methyl bromide.

Concentration	Subjects	Sciatic nerve				Ulnar nerve			
		Base line		End of exposure		Base line		End of exposure	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
65 ppm (subchronic)	Rabbits	42.5	4.4	31.4	3.5	47.8	6.8	35.8	4.9
	Control	40.3	0.9	39.8	2.5	42.0	5.4	41.0	1.5
65 ppm (subchronic)	Rats	49.0	4.5	47.9	6.0	44.8	3.8	42.6	3.6
	Control	44.0	3.4	42.2	1.7	45.2	4.5	47.2	2.7
55 ppm (chronic)	Rats	41.3	3.8	40.8	5.6	42.4	4.1	38.0	6.0
	Control	43.8	5.6	40.5	2.9	44.5	5.3	40.0	5.6

Exposure to 65 ppm of methyl bromide for four weeks produced a marked reduction in the mean amplitude of the eyeblink reflex, equal to about a 50 % loss (fig 3). This effect was apparent in each exposed animal prior to the second week of exposure, and it was progressive in four of the six exposed animals so that, by the last week of exposure, their average loss approached 65 %. The controls displayed a decline similar to that seen in the exposed animals at the end of the first week of the exposure period, but this decline was reversed in the following week in the controls. An analysis of variance, conducted on the mean eyeblink amplitudes of each animal, indicated a significant effect over the course of the exposures ($F = 8.35$, $df = 4 \text{ \& } 20$, $p <$

0.001), and a comparison of the results from the first exposure test with the results from the baseline test revealed a significant decrement in reflex amplitudes by the end of the first week of exposure ($t = 5.03$, $p < 0.005$).

The rats in both the chronic (1,080 h) exposure to 55 ppm and subchronic (100 h) exposure to 65 ppm of methyl bromide and their comparable controls exhibited no change in weight or grip/coordination over the course of the experiment. During the period of chronic exposure, 3 (of 8) control rats and 6 (of 32) exposed rats died of undetermined causes. None died during the subchronic exposures.

There were no significant changes in activity levels between the control and exposed groups in the 12 months of 55-ppm

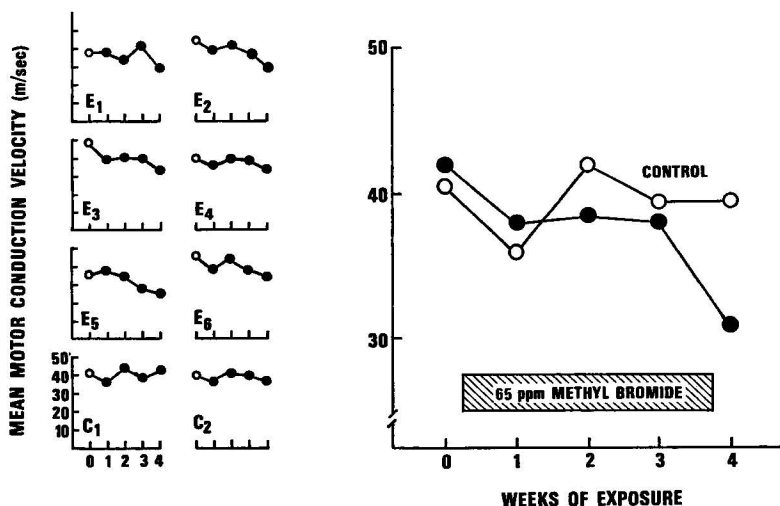


Fig 2. Mean sciatic conduction velocity for rabbits exposed to 65 ppm of methyl bromide and for controls over the same period of time. Individual data are presented in the left panel, and group means are in the right panel.

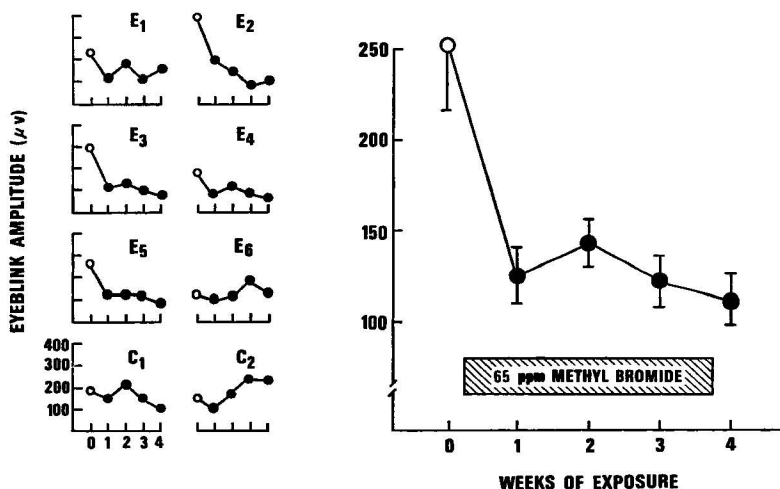


Fig 3. Mean eyeblink amplitude (in microvolts displacement) of rabbits exposed to 65 ppm of methyl bromide for four weeks. Individual data are presented in the left panel, and the group means are in the right panel.

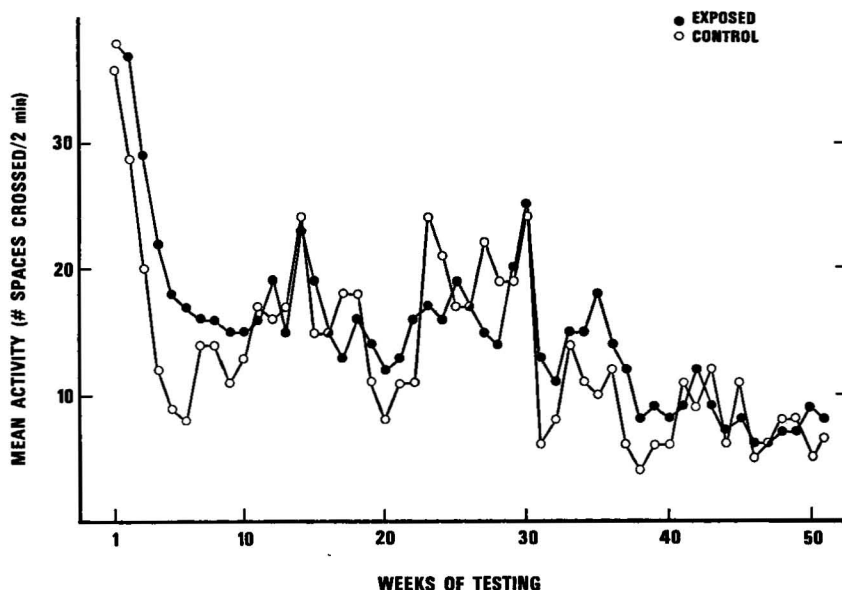


Fig 4. Number of segments entered by rats in the open field activity test during the 51 weeks of the experiment with chronic 55-ppm exposure (weeks are included when equipment malfunction prevented exposures).

exposure. In the chronic 55-ppm exposure test series, the mean number of squares entered over the course of the study was 13 for the control subjects and 15 for the methyl bromide-exposed rats. Weekly activity measures are presented in fig 4 for these animals. As is evident in the figure, there was a gradual decline in activity in both the control and methyl bromide-exposed subjects over the first month of the exposure, but the pattern of decline for both the exposed and control animals was similar, and this trend also occurred for rats exposed to 0 or 65 ppm of methyl bromide. The rats exposed to 65 ppm of methyl bromide entered a smaller number of squares (6) than did the rats exposed to 55 ppm or their controls (on a comparable number of tests). However, differences in animal ages and test conditions possibly account for the disparity.

In the 55-ppm chronic exposure experiment with rats, the mean conduction velocity in the sciatic and ulnar nerves declined in both the experimental and control subjects over the course of the study, but the changes were within one standard deviation of the baseline values (table 1). The slight differences seen in the final test session between the con-

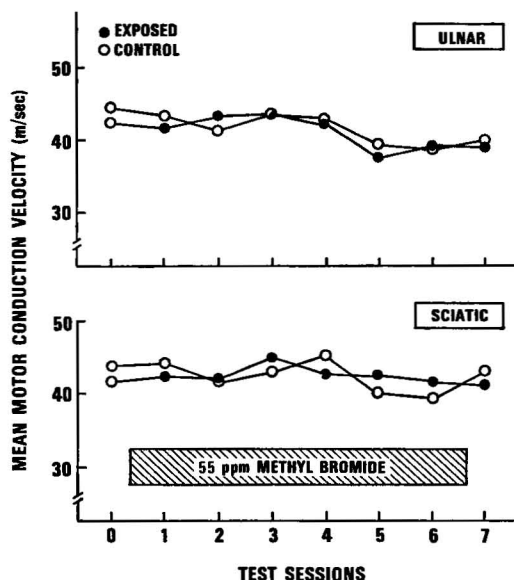


Fig 5. Mean sciatic and ulnar conduction velocity for the experimental and control groups of rats from the baseline period and the seven tests during exposure to 55 ppm of methyl bromide.

trol and experimental groups for the sciatic ($t = 0.52$, $p = 0.61$) and ulnar nerves ($t = 0.70$, $p = 0.49$) were not significant. Fig 5 presents the mean sciatic and ulnar conduction velocity for the ex-

perimental and control groups of the seven conduction tests over the course of the exposure period, plus the baseline test prior to exposure.

In the one-month exposure to 65 ppm of methyl bromide, the mean sciatic conduction rates declined by 1.8 m/s for the control rats compared with a 1.1 m/s decline for the exposed rats. Comparable ulnar changes were a 2.0 m/s increase for the controls and a 2.2 m/s decrease for the exposed subjects, or about one standard deviation. A paired *t*-test run on baseline versus week 4 conduction velocity was not significant for the sciatic ($t = 0.50$, $p = 0.62$) or ulnar ($t = 1.47$, $p = 0.16$) nerve. An independent *t*-test was also run between the control and exposed rats for both sciatic ($t = 1.63$, $p = 0.12$) and ulnar ($t = 2.43$, $p = 0.03$) nerve conduction velocity at the conclusion of exposure. Their significance is doubtful in light of the fact that initial differences between the controls and the (to be) exposed rats were revealed during baseline testing and the change between the baseline and week 4 exposure was minimal. The higher overall nerve conduction velocity in these rats (compared to the rats in the 55-ppm exposure) cannot be satisfactorily explained, although past experience in our laboratory suggests that the relatively small number of animals tested may have resulted in a sampling error.

Discussion

A one-month exposure to 65 ppm of methyl bromide had profound effects on the rabbits; it produced losses in weight, changes in locomotion, and statistically significant decreases in eyeblink reflex magnitude and nerve conduction velocity. The sciatic nerve deficits seen in the present study agree with the report by Irish et al (10) of the development of hindlimb paralysis in rabbits after 3–8 weeks of exposure to 65 ppm of methyl bromide, an effect which was further confirmed in a recent NIOSH contract final report, by Sikov et al (unpublished results), on rabbits after one week of 70-ppm exposure. The significant changes in the ulnar nerve conduction velocity in the present paper extend this finding by suggesting that rab-

bbits exposed to 65 ppm of methyl bromide also exhibit forelimb deficits, an effect rarely reported in conjunction with peripheral neuropathy. The impairment of the eyeblink reflex, evident by the second week of methyl bromide exposure, is consistent with Sikov et al's report of the development of hindlimb paralysis after only one week of exposure to 70 ppm, although the latter finding cannot be reconciled with the onset of paralysis seen in 1940 (10) by Irish et al (< 3 weeks) or in the present study (4 weeks). Taken together, the results of Irish et al (10), Sikov et al (unpublished results), and the present experiment suggest strongly that, in the rabbit, motor mechanisms of diverse origin may be particularly vulnerable to the early untoward effects of methyl bromide exposure. The onset of eyeblink reflex impairment in rabbits two weeks prior to the development of nerve conduction reductions and loss of limb control suggests that certain neurobehavioral changes may precede the peripheral changes in lower (and now in upper) limb functioning reported for this compound. Replications to verify and extend this finding are now underway in our laboratories.

The lack of effect in rats following a chronic exposure to 55 ppm or subchronic exposure to 65 ppm of methyl bromide agrees with the Irish et al report (10) that these levels do not produce frank peripheral neuropathy in rats. The present study, in fact, extends this earlier finding by providing objective and quantitative evaluations of nerve conduction velocity, rather than relying on the subjective methods available in 1940, and it is the first true chronic exposure to be reported for animals. While a recent study has reported behavioral changes in rats following a three-week exposure to 300 ppm of methyl bromide (8), evidence of peripheral neuropathy has not been seen in the rat following methyl bromide exposure. Furthermore, the lack of effect of 65 ppm on rats in the present study, when compared to the profound effects on rabbits at the same concentration, confirms the report of species differences by Irish et al (10), again, using more quantitative evaluations of peripheral neurological impairment.

Disclaimer

Mention of product or company names in this paper does not constitute endorsement by the National Institute for Occupational Safety and Health.

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